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Cord Blood IgM, Triiodothyronine (T_3), T_4 and TSH : Neonatal Thyroid Activity in Intrauterine Infection

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Abstract : Elevated levels of cord blood IgM are non-specific indicators of intrauterine infections. Preliminary experiments showed that when cord blood IgM was elevated, the level of triiodothyronine (T_3) in cord blood was also elevated. This study aimed to evaluate neonatal thyroid function in situations where cord blood IgM is elevated. Neonatal thyroid activity was assessed by the determination of cord blood T_3, T_4 and TSH.

Two groups of infants were selected by screening those born to 346 randomly selected women. One group had cord blood IgM levels greater than 20 mg/100 ml and the other group did not have detectable IgM in cord blood. In the group with IgM greater than 20 mg/100 ml, the levels of T_3 and TSH were significantly higher than the control group. The results indicate an alteration of thyroid activity in situations where cord blood IgM is elevated.

1. Introduction

We have previously demonstrated an association between elevated levels of cord blood IgM and T_3.[10] Although possible intrauterine infection was considered as a likely cause, the limited information available at the time did not permit the evaluation of the neonate's thyroid activity in relation to elevated cord blood IgM.

In the present investigation, the thyroid activity of a group of neonates with raised levels of cord blood IgM was studied in comparison with a normal group. Cord serum T_3, T_4 and TSH levels were considered as indices of neonatal thyroid activity. The possible interferences due to leakage of maternal serum components into the foetal circulation and also the effects of abnormal pregnancy and delivery were taken into consideration.

2. Materials and Methods

2.1 Pregnant women, neonates and collection of cord blood

346 pregnant women admitted to the 'non-paying' wards of the General Hospital, Kandy, were studied. Informed consent was obtained in all cases. Venous blood (6 ml) was collected and serum stored at -20°C until used. Information regarding maternal age, parity, attendance at antenatal clinics and gestational age calculated from last regular menstrual period was recorded. The clinical examinations were particularly directed towards finding any evidence of systemic infections during pregnancy.

The neonates born to these women were the test subjects. Information on the nature of delivery (as normal, artificial rupture of membranes, use of forceps or Caesarian section), birth weight, sex, presence of congenital malformations and multiple pregnancy was collected.

Umbilical cord blood was collected within two minutes of birth. Extreme care was taken to avoid contamination with maternal blood. The level of cord blood IgM was determined in all cases.

From among the full term neonates with a satisfactory birth weight, two groups were selected on the basis of cord blood IgM content.

Group 1: Neonates born to mothers with a normal pregnancy but with cord blood IgM greater than 20 mg/100 ml (approximately 24 IU/ml), N = 29.

Group 2: Gestational age and birth weight matched neonates (to those of Group 1) but without any detectable IgM in cord blood, N=33. (Lower limit of IgM detection, 0.84 mg/100ml).

2.2 Determination of serum protein levels

Total protein levels in serum were determined by the method of Lowry *et al*[16] using purified bovine serum albumin as standard. The levels of IgM and IgG were determined by Single Radial Immunodiffusion (SRID) in 1.5% agarose using class specific antisera obtained from Nordic Immunological Laboratories, Netherlands. Determination of transferrin and Pregnancy Zone Protein was also by SRID. The latter was performed according to the method of von Schoultz.[26]

2.3 Determination of cord serum T_3, T_4 and TSH levels

Radioimmunoassay methods employing specific antisera were used with 8 aniline naphthalene sulphonic acid (ANS) added to the test serum to inhibit the binding of hormone to endogenous protein. The method of Eastman *et al*[12] was used to measure T_3 and that of Corcoran[7] for T_4.Routine quality control samples obtained from the World Health Organization were used in all assays. Specific antisera to T_3 and T_4 were gifts from Professor J. Kosowicz, Medical Academy, Pozanan, Poland. ^{125}I–T_3 (T_3 tracer, specific activity 60 Ci/g) and ^{125}I–T_4 (T_4 tracer, specific activity 2300 Ci/g) were obtained from the Radiochemical Centre, Amersham, England. The sensitivities of the assays (as precision of determination of zero hormone concentration) were 0.015 nmol/l of T_3 and 3.86 nmol/l of T_4. Within assay variation for T_3 was 6% at 0.79 nmol/l and 8% at 3.07 nmol/l. The corresponding values for T_4 were 5% at 64.35 nmol/l and 6% at 257 nmol/l. Between assay variation for (12 samples in duplicate in 5 assays) was 11% for T_3 and 13% for T_4. Non-specific binding was below 10% for T_4 and 13% for T_3.

The TSH Test Kit (Phadebas) of Pharmacia Diagnostics AB, Uppsala, Sweden, was used for the determination of TSH levels.

2.4 Determination of thyroid microsomal antibodies

Antibody to thyroid microsomal antigen in maternal and cord serum was determined by haemagglutination using the 'Microsome Test Kit' of Fujizoki Pharmaceutical Co. Ltd., Tokyo, Japan.

3. Results

The mean ± S. D. of maternal age, weight, parity, total serum proteins and serum transferrin for mothers of Group 1 infants were 28.8 ± 6.8 years, 51.4 ± 7 kg, 3.6 ± 2.9, 7.01 ± 0.7 g/100 ml and 370 ± 90 mg/100 ml respectively. The corresponding values for Group 2 were 29.13 ± 5.3 years, 51 ± 6.7 kg, 3.8 ± 2.2, 7 ± 0.7 g/100 ml and 380 ± 67 mg/100 ml. None of these values was significantly different in the two groups.

IgM was absent in 207 cases while 35 infants had IgM greater than 20 mg/100 ml (approximately 24 IU/ml). The upper limit of the normal range of cord blood IgM was taken as 20 mg/100 ml.[24]

The mean ± S. D. of birth weight, gestational age, cord serum levels of IgG, IgM, transferrin, T_3 T_4 and TSH of infants of Group 1 and Group 2 are shown in Table 1. The mean birth weights, gestational ages and the levels of IgG and T_4 were not significantly different in the two groups. The level of transferrin was lower in Group 1 than Group 2. The levels of IgM, T_3 and TSH were significantly higher in Group 1 than Group 2. The ratio T_4/T_3 was in favour of T_3 in Group 1.

TABLE 1. Mean ± S. D. of Birth Weights, Gestational Ages and Cord Serum Levels of IgG, IgM, T_3, T_4, TSH and Ratio T_3/T_4 in Neonates of Group 1 & 2.

Neonates	Gestational age (weeks)	Birth Weight (kg)	IgG mg/ 100ml	IgM, mg/ 100ml	T_3 (nmol/l)	T_4 (nmol/l)	TSH (mU/l)	T_4/T_3
Group 1	39.8 ± 0.8	2.7 ± 0.36	1365 ± 354	23 ± 8	0.64 ± 0.18	100 ± 30	23 ± 20	162 ± 49
Group 2	39.6 ± 1.6	2.8 ± 0.44	1217 ± 340	N.D.	0.49 ± 0.2	93 ± 24	11 ± 7	240 ± 181
Significance of the difference.	t = N. S. P =	N. S.	N. S.	**	3.463 0.001	N. S.	3.307 0.01	2.3 0.05

N.D. ; Not Detectable, N.S. ; Not Significant.

** Significance level cannot be determined as the absolute values for Group 2 could not be assigned.

Antibodies to thyroid microsomal antigen were seen in two mothers and four neonates. In the two cases where both maternal and cord blood were positive, the antibody titres were similar (range 1:200 to 1:400) and the two infants whose maternal blood was negative, the titre of antibody was 1:400. These four infants belonged to Group 1. None of the infants in Group 2 had antibody to thyroid microsomal antigen in their cord blood.

4. Discussion

The results of the present investigation, while confirming the earlier report[10] demonstrate raised T_3, TSH and normal T_4 levels in cord blood of neonates who had elevated levels of IgM ; to our knowledge a hitherto unpublished observation.

Since IgM does not cross the placenta and the possibility of contamination of cord blood with maternal blood was excluded (see below), the IgM in cord blood of neonates in Group 1 was probably due to foetal synthesis. The normal foetus has the potential to synthesize IgM[24] and raised levels of IgM in cord blood (greater than 20 mg/100 ml, approximately 24 IU/ml) have been shown to be a non-specific indicator of intrauterine infections.[1,2,24] Furthermore, specific IgM antibody to filarial antigens were seen in 10 neonates[11] and toxoplasmosis antibody was seen in 8 cases (unpublished), all being of Group 1. Therefore, it was concluded that neonates of Group 1 suffered from subclinical infection during intrauterine life.

As post term delivery, foetal distress and Caesarian section, etc., are known to affect cord blood TSH levels [25] care was taken to include only normal healthy infants born to mothers with a normal pregnancy in the series. Also, prenatal records did not indicate any evidence of foetal undernutrition during pregnancy. The collection of cord blood during the first two minutes after delivery excluded the possibility of post natal TSH surge.[13]

Contamination of cord blood with maternal blood during collection was excluded on the basis of pregnancy zone protein and transferrin levels. The pregnancy zone protein does not cross the placenta and is absent in cord blood.[3] None of the cord blood samples included in this study had detectable levels of pregnancy zone protein. Also, the mean level of transferrin in Group 1 neonates was lower than that of Group 2 neonates.

The mechanism of elevation of T_3 and TSH (with normal levels of T_4) was probably due to a defect in the regulatory processes of T_3 and TSH secretion. As the mean levels of birth weights and the gestational ages were not different in the two groups, the observed elevations were not due to differences in foetal maturity.[6] Certain intrauterine infections are known to cause foetal malformations associated with brain damage.[9,14,27] Entry of T_3 into the brain may be important in the regulation of TSH secretion.[4,5,28] Sing-Yung Wu[23] has demonstrated that binding of T_3 to serum

immunoglobulins makes the bound T_3 metabolically less available and an euthyroid state can persist in the presence of relatively high levels of serum T_3. Such a situation could also diminish the entry of T_3 into the brain. The possibility that in intrauterine infections, foetal immunoglobulins could cause such a situation is presently under investigation.

The elevation of T_3 and TSH in iodine deficiency goitre[8,15,17,20] has been explained as due to a preferential secretion of T_4 at the expense of T_3 as part of an adaptive mechanism to produce compensated euthyroidism.[19] It was also possible that the increased T_3 was due to preferential formation of T_3 than rT_3 by the peripheral deiodination of T_4. However, in the present investigation, we were unable to measure rT_3 and this possibility could not be investigated.

The observation that two infants had 'foetal' antibodies to thyroid microsomal antigen (in the absence of this antibody in corresponding maternal blood samples) is of interest. These two infants, on re-examination at 12-18 months of age did not show any evidence of thyroid dysfunction. Even though the maternal thyroid antibody transferred across the placenta do not appear to produce clinical symptoms in the newborn [18,22] and overt thyroid disease is not found in some serologically positive subjects,[21,29] subclinical thyroid damage as suggested by Yoshida *et al*[29] cannot be excluded.

To our knowledge, the presence of foetal antibodies to thyroid microsomal antigens in cord blood has not been reported previously. This observation also raises the fundamental question as to whether a thyroidal autoimmune response is possible in the foetus.

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